PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
PROJECT LEADER: J. M. Penn
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I. INHIBITION OF EGF BINDING ASSAY

A. **Objective**: Evaluate the role of catechol in CSC activity as measured by the EGF assay.

B. **Results**: An experiment was performed to compare the effects of 2R1 CSC dissolved in acetone and methanol since the chromatographic removal of catechol from CSC would require the use one of these solvents. As compared to the standard DMSO solvent, both acetone and methanol produced the same magnitude of inhibition of EGF binding and reduction in cell numbers. Therefore, either solvent can be used without affecting CSC activity in the EGF assay.

Two experiments were conducted to determine the binding kinetics of catechol as compared to 2R1 CSC. Binding analysis indicated that catechol and 2R1 CSC reduced receptor numbers as well as affinity.

C. **Plans**: Conduct experiments using a broader range of EGF concentrations to distinguish between a change in affinity and the loss of high affinity receptor sites.

D. **Reference**:

Patskan, G. Notebook No. 8738, p. 31.

II. SALMONELLA/MICROSOME (S/M) ASSAY -- CROSSED SOLUBLES/BASE WEB STUDY

A. **Objective**: To determine if there is a concentration effect during the ultrafiltration process of BuS1 which has been retained and passed through a 1000 MW filtration membrane.

B. **Results**: BuS1 was carried through the ultrafiltration process at various concentrations (0.1-0.5 mg/ml). The results showed that none of the test samples were significantly different from the BuS1 control. There was also no distinct concentration effect for either of the material that passed through or that which was retained.

C. **Plans**: There are no immediate plans to continue work on ultrafiltration.

D. **Reference**:

Thompson, L. H. Notebook No. 8731, pp. 1-14.

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III. S/M ASSAY -- EVALUATION OF D-ALANINE

A. **Objective**: Determine the activity of d-alanine as a pure compound in the S/M assay.

B. **Results**: D-alanine was evaluated using the standard S/M protocol for pure compounds in the presence and absence of S9 in strains TA98 and TA100. The results indicated that there was no S/M activity of d-alanine under any test condition. In addition, there was no evidence of toxicity at any dose tested.

C. **Plans**: Evaluate 5 and 10 mM of d-alanine oversprayed onto 1R4F using water as the solvent.

D. **Reference**:

Thompson, L. H. Notebook No. 8731, pp. 15-20.

IV. PROTEIN KINASE C (PKC) IN INTACT CELLS

A. **Objective**: To determine the dose response effects of CSC in the PKC assay.

B. **Results**: Two types of CSCs (2R1 and CDL) were evaluated in the PKC assay. The results indicated that both CSCs produced qualitatively similar patterns of phosphorylation. However, there still appears to be difficulties with experimental variability. Several protocol modifications were implemented to improve reproducibility, but a significant amount of variability remains.

C. **Plans**: Conduct experiments to determine if experimental variability can be eliminated.

D. **Reference**:

Nixon, G. M. Notebook No. 8711, p. 46.

V. GLUTATHIONE DEPLETION ASSAY (GDA)

A. **Objective**: To determine if CDNB or 2NF can be used as a control compound to examine the effects of 2R1 CSC induced reduction in GSH levels on TA98 mutagenic activity.

B. **Results**: Two experiments were carried out to determine the effects of 2R1 CSC and CDNB pretreatment on GSH levels and TA98 activity of CDNB. 2R1 CSC at a dose of 0.2 mg/plate produced a 25% reduction in the GSH level, while CDNB at a dose of 50 µM reduced the GSH level by 97%. Pretreatment of cells with 2R1 CSC or CDNB had no effect on the TA98 mutagenic activity of CDNB. A third experiment was conducted to determine the effects of 2R1 CSC and added GSH on the TA98 activity of 2NF and CDNB. Each tested in the presence of CSC (0.2 mg/plate) showed a reduction in activity (50% and 30% respectively). When each was tested in the

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presence of CSC and added GSH (2.5 mM) a further reduction in activity (14% and 13%, respectively) was noted.

C. **Plans**: Determine the effects of added GSH and added NADP on the TA98 activity of 2NF and CDNB.

D. **Reference**:

McCoy, W. R. Notebook No. 8739, pp. 5-9.

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